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Poly(2-oxazoline) Molecular Brushes by grafting through of poly(2-oxazoline)methacrylates with aqueous ATRP

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Keywords: atom transfer radical polymerization (ATRP), controlled radical polymerization, molecular brush, poly(2-oxazoline), stimuli responsive polymer

Abstract

Molecular brushes of poly(2-oxazoline)s (POx) are an intriguing class of polymers as they combine a unique architecture with the properties of POx as a biomaterial. Here, the synthesis of several POx macromonomers with methacrylate end groups and consecutive grafting through polymerization by aqueous atom transfer radical polymerization (ATRP) at room temperature is reported. ^{1}H-NMR spectroscopy and size exclusion chromatography (SEC) confirmed the synthesis of POx molecular brushes with maximum side chain grafting densities, narrow molar mass distributions ($Đ \leq 1.16$) and final molar masses corresponding to the initial macromonomer : initiator ratio. Chain extension experiments show high end group fidelity, formation of block copolymer molecular brushes and kinetic studies revealed a polymerization behavior of oligo(2-methyl-2-oxazoline methacrylate) very similar to the frequently used oligo(ethylene glycol) methacrylate ($OEGMA_{475}$). Aqueous solutions of POx molecular brushes with poly(2-ethyl- and 2-isopropyl-2-oxazoline) side chains exhibit the typically defined thermoresponsive behavior with a tunable, very narrow and reversible phase transition.

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Introduction

Molecular brushes are composed of a macromolecular backbone with a maximum number of pending polymeric or oligomeric side chains. The very high grafting density induces side chain as well as backbone stretching which results in a characteristic elongated, worm-like shape of the molecular brush.[1] The synthesis of molecular brushes is classified into three methods of whom every single one has its specific advantages. These methods are *grafting onto* (synthesis of side chains and backbone with a subsequent coupling), *grafting from* (polymerization of side chains starting from a macroinitiator backbone) and *grafting through* (polymerization of macromonomers).[1] The unique structure, considerable size and high aspect ratio of molecular brushes make them suitable for the use as single molecule nanomaterials to be used as templates, actuators and sensors in nano(bio)technology[2–4] or even in nanomedicine as drug-delivery systems.[5,6]

Molecular brushes based on poly(2-oxazoline)s (POx) got in focus of recent research because of the advantageous properties of POx as a biomaterial. POxs are pseudo-peptides synthesized by living cationic ring-opening polymerization (LCROP) of 2-oxazolines in a controlled manner ($Đ < 1.2$, adjustable molar masses).[7,8] Similar to poly(ethylene glycol) (PEG), hydrophilic POx is non-toxic,[9,10] non-immunogenic (low to none complement activation),[10,11] suppresses biofouling,[12,13] POxylated entities display the same "stealth effect" as PEGylated ones,[14–16] and hydrophilic as well as amphiphilic POx shows a biodistribution and excretion which is beneficial for medical applications.[17] Additionally their solubility and aggregation behavior can be fine-tuned by structural and compositional variation of the poly(2-oxazoline)s by usage of easy accessible monomers.[8,18–20] Furthermore, multiple functionalization and structural versatility is possible using respective initiators, monomers and terminating agents.[7,21–30] After early approaches[31,32] yielding a variety of POx-based comb polymers,[33–46] POx molecular brushes have been synthesized recently by several routes. The majority of the applied methods focuses on the polymerization of macromonomers by *group transfer polymerization* (GTP), *free radical polymerization* (FRP) or *reversible addition/fragmentation chain transfer polymerization* (RAFT). GTP and RAFT can result in molecular brushes with narrow molar mass distributions, but are still limited to relatively short backbones and side chains.[31,47,48] Unfortunately, GTP is demanding with respect to the strict reaction conditions and the more robust RAFT has to be stopped at rather low conversions (usually ~50 %) in order to suppress side reactions.[48]

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Nevertheless, RAFT has successfully been used for the synthesis of copolymer molecular brushes made from short ($P_n = 5$) 2-ethyl- and 2-nonyl-2-oxazoline macromonomers.[49] FRP of macromonomers results in molecular brushes with a very long backbone ($P_w \sim 1050$) but a broad molar mass distribution.[50] Alternatively, in different *grafting from* approaches, 2-isopropenyl-2-oxazoline was polymerized using FRP, anionic polymerization or rare earth metal catalyzed GTP to create a backbone from which different 2-oxazolines were polymerized *via* living cationic ring-opening polymerization (LCROP) to yield molecular brushes.[51,52] The anionic polymerization resulted in defined brushes, even with block copolymer side chains, but again under very demanding reaction conditions. The rare earth metal catalyzed GTP is stated to perform better, but only indicated by AFM-imaging so far.[52] In addition, the rare earth metal catalyst is rather difficult to handle. One of the most interesting features of POx molecular brushes is their defined thermoresponsive behavior. The temperature depended solubility in water can be fine-tuned by varying the length of the backbone, the side chain or the side chain composition.[48,50,51,53–56] Thus, POx molecular brushes are interesting materials to be used as sensors or, with a cloud points adjustable close to the human body temperature, for biomedical applications such as theragnostics. Furthermore, it is possible to conjugate antibodies to molecular brushes of 2-ethyl- and 2-isopropyl-2-oxazoline, which could be interesting for the development of active targeting in drug-delivery.[57]

As outlined above, the existing methods for the synthesis of POx molecular brushes are either experimentally demanding or uncontrolled and/or limited to rather short side chain or backbone lengths. Here, the *atom transfer radical polymerization* (ATRP) should be an attractive alternative to overcome these drawbacks. However, first attempts to use ATRP for the synthesis of POx molecular brushes were done by ATRP of 2-isopropenyl-2-oxazoline and subsequent LCROP of 2-oxazolines, thus, a typical *grafting from* approach. Unfortunately, ATRP of 2-isopropenyl-2-oxazoline yielded only oligomeric products, which was contributed to a strong complexation of the copper species used for the ATRP catalyst by the monomer and the growing oligomers.[55] To the best of our knowledge, ATRP of POx macromonomers has not been attempted so far and could be more viable. For example, similar oligo(ethylene glycol) methacrylate can be polymerized by aqueous ATRP in a fast and well-controlled manner. Even grafting from a protein is possible.[58,59] Moreover, the synthesis of suitable POx macromonomers is well known and reported in the literature.[31,37,48,60–62]

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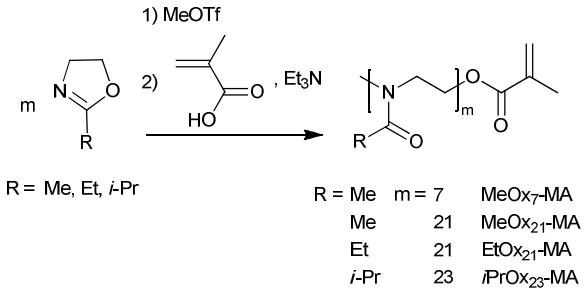
Here we report on the well-controlled synthesis of POx molecular brushes by the *grafting through* of poly(2-oxazoline)methacrylates by aqueous ATRP.

Results and Discussion

Synthesis of poly(2-oxazoline)methacrylate macromonomers

Defined POx macromonomers were synthesized according to Kobayashi *et al.*[60] by LCROP of 2-methyl- (MeOx), 2-ethyl- (EtOx) or 2-isopropyl-2-oxazoline (*i*PrOx) with methyl triflate as the initiator at 90°C in acetonitrile (Scheme 1). The LCROP was terminated by a mixture of methacrylic acid and triethylamine creating the termination agent, a methacrylate anion, *in situ* to yield the methacylate end functionalized POx ($MeOx_m$-MA, $EtOx_m$-MA and *i*$PrOx_m$-MA). Analog to oligo(ethylene glycol) macromonomers, short POx macromonomers with m = 7 but also longer ones with m = 21, 23 were synthesized to investigate their consecutive polymerizability by ATRP. The length of the macromonomers was controlled by the initial $[M]_0$:$[I]_0$ ratio. While MeOx yields highly water-soluble polymers, EtOx and especially *i*PrOx give thermosensitive polymers.[63–68]



Scheme 1. Synthesis of poly(2-oxazoline) macromonomers by LCROP with methyl triflate as the initiator and methacrylic acid / triethylamine termination.

The four POx-MA macromonomers were analyzed by means of SEC, matrix assisted laser desorption ionization - time of flight mass spectrometry (MALDI-ToF-MS) and ^{1}H-NMR spectroscopy. With the latter, the chemical composition of all macromonomers could be confirmed, especially the methacrylic end function by the appearance of two singlets around 5.8 ppm corresponding to the two vinylic protons (Figure 1). Comparison of the peak integral ratio of the terminal methyl group originating from the initiator (two multiplets between 2.8 and 3.1 ppm) to the methacrylic group indicates a quantitative functionalization by the termination reaction. The ^{1}H-NMR spectrum also shows minor traces of diethyl ether (from precipitation) and

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triethylammonium ions (from the termination reaction). The impurities could be removed by dialysis, however, because of the relatively low molar mass of the macromonomers, this also caused a significant loss of product and since both impurities are not interfering with the consecutive ATRP reaction, the macromonomers were used as such for further experiments. The presence of triethylammonium salt caused obstruction in the SEC trace of the low molar mass $MeOx_7$-MA as both compounds had a similar elution time. This resulted in a relatively high apparent dispersity of $Đ_{SEC}$ = 1.31 while all other macromonomers gave dispersity values of $Đ_{SEC}$ = 1.08-1.09 and thus, close to the theoretical limit (Figure 2a, Table 1). This is also corroborated by the MALDI-ToF-MS data as only molar mass distributions corresponding to the desired macromonomer structure of poly(2-methyl-2-oxazoline) ($\Delta m/z$ = 85), poly(2-ethyl-2-oxazoline) ($\Delta m/z$ = 99) or poly(2-isopropyl-2-oxazoline) ($\Delta m/z$ = 113) with a methyl group at the one and a methacrylic group at the other chain end can be observed in the MALDI-ToF-MS spectra (Figure 2b and 2c). A straightforward analysis of the short $MeOx_7$-MA is again obstructed only this time, by the overlapp of the mass signals of the macromonomer with matrix components. Nevertheless, close inspection of the mass spectrum indicate a much better dispersity of $MeOx_7$-MA as calculated from the deformed SEC elugram.

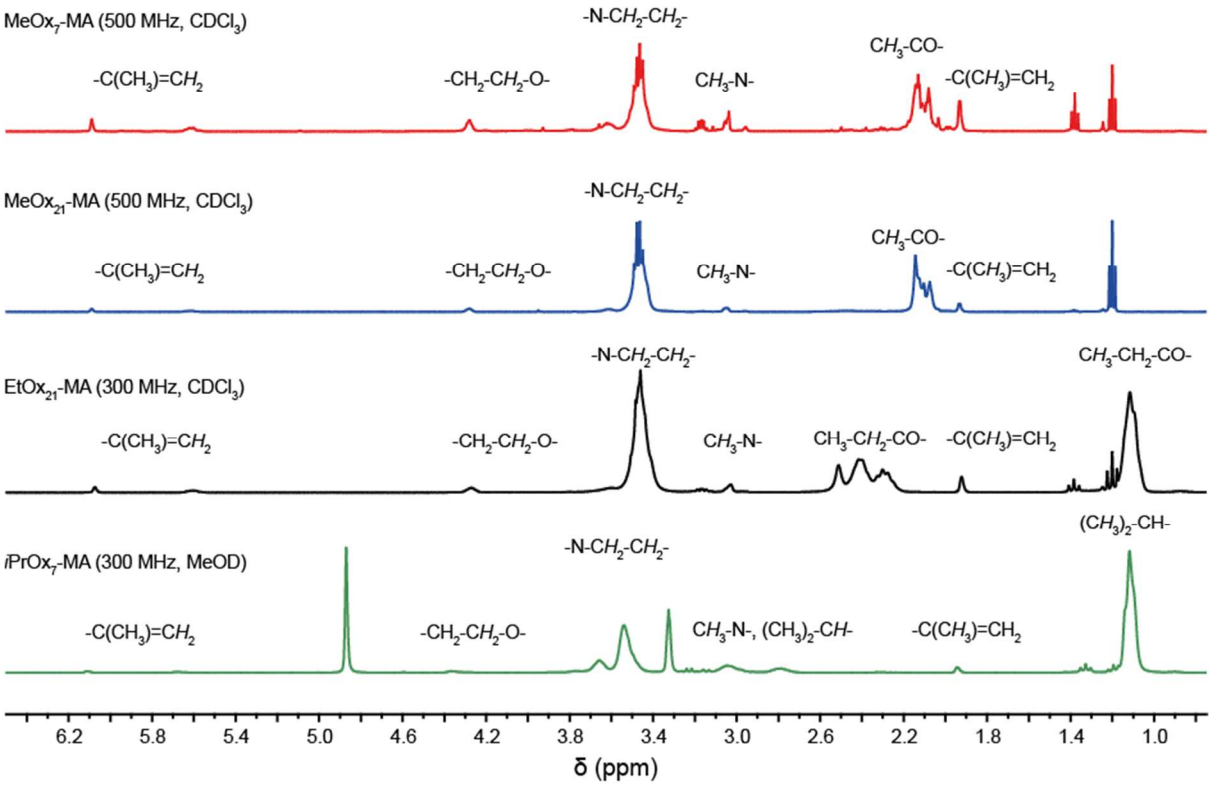

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Figure 1. [1]H-NMR-spectra of the synthesized macromonomers with signals aligned to the respective structural units.

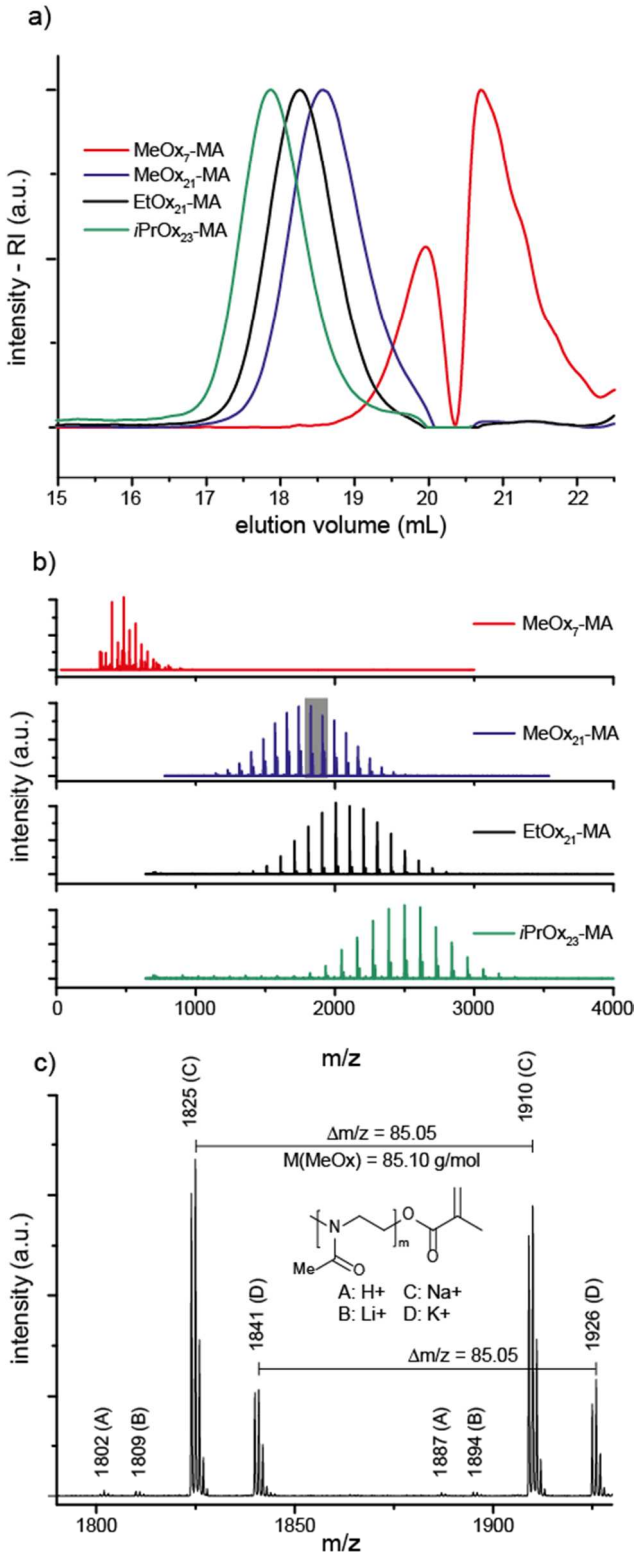

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Figure 2. Characterization of the POx-MA macromonomers. a) SEC elugrams of the macromonomers showing the narrow molar mass distribution and the growth of the molar mass with increasing side chain length and degree of polymerization. b) MALDI-ToF-MS spectra of the macromonomers demonstrating narrow molar mass distribution and only the desired macromonomer species. Distribution of $MeOx_7$-MA is overlapped by peaks of the matrix. c) Detailed section of the spectrum of $MeOx_{21}$-MA. The chosen part is highlighted (grey area) in b).

Table 1. Synthesis details and analytical data of the POx_m-MA macromonomers.

Name	R	$[M]_0/[I]_0$	$M_{theo.}$[a] (g/mol)	$P_{n,NMR}$[b]	$M_{n,NMR}$[b] (g/mol)	$M_{n,MALDI}$[c] (g/mol)	$M_{n,SEC}$[d] (g/mol)	$Đ_{SEC}$[d]
$MeOx_7$-MA	Me	5	525	7	695	524	424	1.31[e]
$MeOx_{21}$-MA	Me	20	1802	21	1887	1790	2010	1.09
$EtOx_{21}$-MA	Et	20	2083	21	2182	2050	2484	1.08
i$PrOx_{23}$-MA	*i*-Pr	20	2363	23	2703	2450	3063	1.08

[a] As calculated from $[M]_0$:$[I]_0$. [b] As determined by endgroup analysis form ^{1}H-NMR spectroscopy data. [c] As determined by MALDI-ToF-MS. [d] As determined by SEC (DMAc, 5 g/L LiBr, 1 vol% H_2O, calibrated with PMMA standards). [e] Apparent dispersity obtained from obstructed elution peak as shown in Figure 2a. The actual dispersity is significantly lower.

Synthesis of POx molecular brushes

We tested several ATRP systems with different ligands (*N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA), tris[2-(dimethylamino)ethyl]amine (Me_6TREN), 2,2'-bipyridine (bpy)), with or without initial addition of a deactivating copper(II)-complex to polymerize the POx macromonomers to molecular brushes (Table 2).

Table 2. Experimental conditions for the ATRP of $MeOx_7$-MA in aqueous solution at room temperature with different ligands and Cu(II) ratio. Reaction time was set to 2 h, aimed degree of polymerization was 30.

#	Ligand	[I] (mM)	Conv. (%)[a]	[M]:[I]:[Cu(I)]:[Cu(II)]:[L]	$M_{n,theo}$ (kg/mol)[b]	$M_{n,SEC}$ (kg/mol)[c]	$Đ_{SEC}$[c]
1	PMDETA	38	86.7	28 : 1 : 1 : 0 : 2	16.8	23.6	2.83
2	Me_6TREN	35	89.5	30 : 1 : 1 : 0 : 1	18.8	57.8	2.23
3	bpy	35	93.5	30 : 1 : 1 : 0 : 2	20.0	164	1.41
4	bpy	8	>99	30 : 1 : 1 : 9 : 22	20.7	10.2	1.15

[a] As determined by SEC analysis of the unpurified molecular brush. [b] As calculated from initial macromonomer-initiator ratio and conversion. [c] As determined by SEC, DMAc, 5 g/L LiBr, 1 vol% H_2O, calibrated with PMMA standards.

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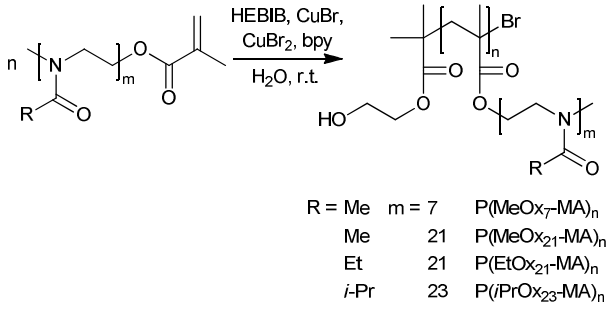
As obvious from Table 2, entry 1 – 3, no controlled ATRP reaction was possible using PMDETA, Me_6TREN or bpy without additional Cu(II) as indicated by the high values for $Đ_{SEC}$ and apparent molar masses being significantly higher than expected. This corresponds well with the literature, where PMDETA and especially Me_6TREN are stated to create highly reactive (the reason they were originally chosen for this study) complexes which disproportionate in aqueous solution, thus creating a more complex reaction system, which in the end can result in high radical concentration and loss of control. Even when using water stable and less reactive bpy complexes initially added Cu(II) species might be necessary to maintain reaction control.[58,69–71] In accordance, the use of 2,2'-bipyridine as the ligand and copper(I)- as well as copper(II)-bromide (Table 2, entry 4) in aqueous solution proved to be a suitable method and results in the desired molecular brushes in high yields, with narrow molar mass distributions and final molar masses in the expected range (Scheme 2). While the initiator, 2-hydroxyethyl-2-bromoisobutyrat (HEBIB), and copper(I)-complex were used in equimolar amounts, a nine fold excess of the deactivating copper(II)-complex was necessary to achieve and maintain satisfying reaction control even at high conversions of the macromonomers. A similar recipe was also successfully applied for the polymerization of oligo(ethylene glycol) methacrylate ($OEGMA_{475}$) by ATRP.[58]



Scheme 2. Synthesis of POx molecular brushes by aqueous ATRP at room temperature from POx macromonomers. The components were typically used at a ratio of [I]:[Cu(I)]:[Cu(II)]:[bpy] = 1:1:9:22.

A series of eleven molecular brushes was successfully synthesized by using this approach and analyzed by means of SEC, NMR and SEC-MALLS (Table 3). In general, conversions > 80 % could be reached within 6 h or less. Residual macromonomer and catalyst could be removed by column chromatography. Figure 3a shows the SEC elugrams of a series of molecular brushes $P(MeOx_7\text{-}MA)_n$ with n = 10 to 820 and the steady increase of the molar masses and low dispersities (Đ = 1.16-1.21) for all brushes. The symmetric SEC traces exhibit neither high nor

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low molecular subpopulation which indicates a fast and quantitative initiation and chain transfer or termination reactions seems not to be significant.

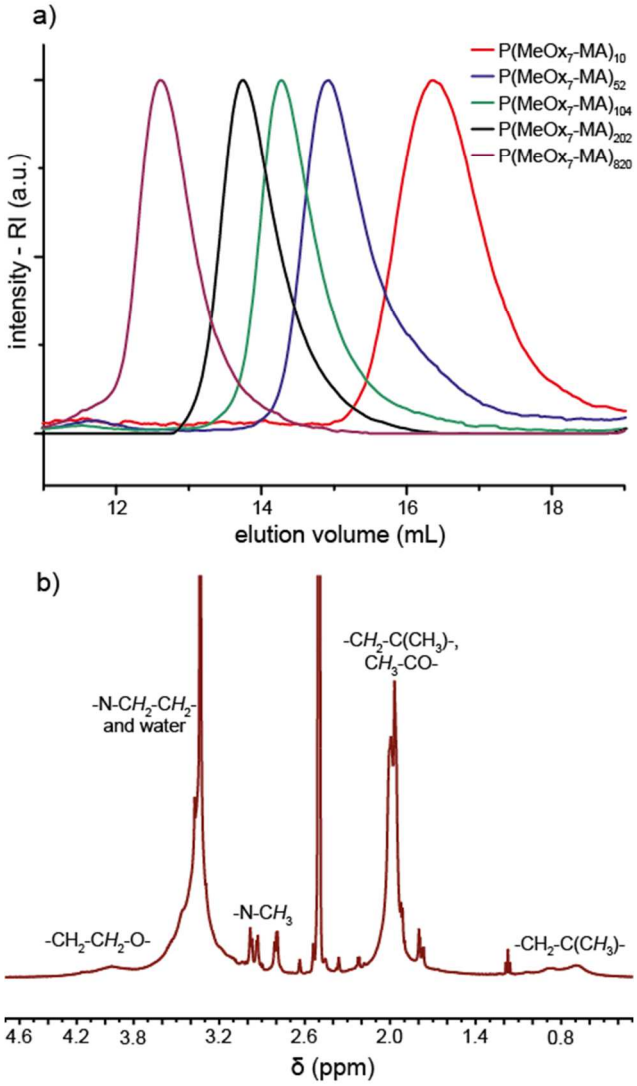

Figure 3. a) SEC elugrams (DMAc, 5 g/L LiBr, 1 vol% H_2O, calibrated with PMMA standards) of five molecular brushes from $MeOx_7$-MA with increasing backbone length. b) ^{1}H-NMR-spectrum (500 MHz, DMSO-d_6) of P($MeOx_7$-MA)$_{52}$ with assignments of the respective structural units.

^{1}H-NMR spectroscopy verifies the anticipated structure of the synthesized brushes. All observable signals can be assigned to the respective structural units as shown exemplary for P($MeOx_7$-MA)$_{52}$ in Figure 3b. Unfortunately, endgroup analysis is not possible because of the very low relative intensity of the terminal hydroxyl group and the broad strong proton signals of the high molar mass brush. The two singlets of the vinylic protons of the macromonomers around

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5.8 ppm are no longer observable and new broad signals between 0.4 to 1.4 ppm arose, indicating the formation of the methacrylate backbone. Comparing the intensities of these backbone signals with signals originating from the side chain terminal methyl groups around 3.0 ppm (3H), a maximum grafting density can be concluded for the series of P($MeOx_m$-MA)$_n$. This analysis was not possible for P($EtOx_m$-MA)$_n$ and P(*i*$PrOx_m$-MA)$_n$ because of signal overlapping (data not shown).

Also longer macromonomers ($MeOx_{21}$-MA and $EtOx_{21}$-MA) could successfully be polymerized to molecular brushes and for all cases the dispersity as determined by SEC ($Đ_{SEC}$) is very low (Table 3). As it can be expected, the number average molar masses of the molecular brushes as determined by SEC deviate from the expected values especially for longer side chain brushes because of the different macromolecular architecture of the used calibration standard being linear PMMA. Nevertheless, an obvious trend is observable with respect to the increasing molar masses of the brushes with the same side chains but increasing backbone length and for comparable backbones but different side chain lengths. While absolute values differ, these trends at least indicate a controlled polymerization and the possibility of adjustable molar masses by ATRP. For better analysis, we performed additional characterization by SEC with light scattering detection (SEC-MALLS, see below).

Naturally, the realizable backbone length of molecular brushes by the grafting through of longer macromonomers is limited by the viscosity of the initial reaction solution. We found that simple dilution, resulted in a noticeable loss of control of the ATRP system as the higher dispersity and deviation of calculated *vs.* determined molar masses for P($MeOx_7$-MA)$_{820}$ and P($EtOx_{21}$-MA)$_{183}$ shows (Table 3). The latter could only be obtained with a double amount of the activator copper complex. Here, alternative controlled/living radical polymerization techniques such as Cu^0-mediated living radical polymerizations might be more useful.[72–75] Related studies are currently ongoing. Nevertheless, aqueous ATRP was found to be a very versatile polymerization to obtain highly defined molecular brushes with considerable side chain and backbone lengths. As the aqueous ATRP can be driven to high conversions it poses a good alternative to other CRP techniques such as RAFT.[48] Only the polymerization of *i*$PrOx_{23}$-MA turned out to be difficult and stopped at a conversion around 66 %. Already the initial reaction solution was very viscous because of the higher molar mass of *i*$PrOx_{23}$-MA and the further significant viscosity increase upon conversion of the macromonomer made stirring impossible.

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Table 3. Experimental and analytical data of the molecular brushes synthesized by aqueous ATRP with [I]:[Cu(I)]:[Cu(II)]:[bpy] = 1:1:9:22; reaction times as listed.

Name	n[a]	[I] (mM)	t_R (h)	Conv. (%)[b]	$M_{n,theo}$ (kg/mol)[a]	$M_{n,SEC}$ (kg/mol)[c]	$Đ_{SEC}$[c]	$M_{n,SEC\text{-}MALLS}$ (kg/mol)[d]
P(MeOx$_7$-MA)$_{10}$	10	5	2	> 99	7.4	6.9	1.16	-[f]
P(MeOx$_7$-MA)$_{52}$	52	5		> 99	36.1	15.5	1.16	40.0
P(MeOx$_7$-MA)$_{104}$	104	5		> 99	72.3	24.2	1.16	65.9
P(MeOx$_7$-MA)$_{202}$	202	5		> 99	140.5	36.3	1.15	101.1
P(MeOx$_7$-MA)$_{820}$	820	1	3	82.1	695.0	93.5	1.21	-[f]
P(MeOx$_{21}$-MA)$_{50}$	50	5	2	86.3	95.1	42.3	1.08	95.7
P(MeOx$_{21}$-MA)$_{91}$	91	2.5	6	86.6	172.4	55.9	1.09	150.1
P(EtOx$_{21}$-MA)$_{50}$	50	5	2	89.2	108.2	48.6	1.06	118.4
P(EtOx$_{21}$-MA)$_{92}$	92	2.5	6	86.0	200.6	71.9	1.08	212.6
P(EtOx$_{21}$-MA)$_{183}$	183	1[e]	5	90.9	398.7	84.4	1.10	276.6
P(*i*PrOx$_{23}$-MA)$_{37}$	37	5	2	65.4	99.2	46.4	1.09	-[f]

[a] As calculated from initial macromonomer-initiator ratio and conversion. [b] As determined by SEC analysis of the unpurified molecular brush. [c] As determined by SEC, DMAc, 5 g/L LiBr, 1 vol% H_2O, calibrated with PMMA standards. [d] As determined by SEC-MALLS, H_2O, 0.72 g/L NaN_3, 8.5 g/L $NaNO_3$, 35 °C. [e] [I]:[Cu(I)]:[Cu(II)]:[bpy] = 1:2:9:25. [f] Not determined due to low amount of polymer or because of polymer - column interaction.

Further analysis of the POx molecular brushes by SEC-MALLS revealed molar masses, which corresponded well with the theoretical values in the majority of the cases and corroborates the assumption of a controlled grafting through polymerization. The traces obtained from SEC-MALLS have a symmetrical appearance and again narrow molar mass distributions can be found. Dispersities range even below ($Đ \leq 1.07$) the values determined by SEC with refractive index detection probably because of the lower scattering of the lower molar mass fraction. The molar mass of P(*i*PrOx$_{23}$-MA)$_{37}$ could not be determined because of interaction of the polymer with the stationary phase at 35°C (standard condition) as well as 25 °C. Higher temperatures could not be used because of the cloud point of the polymer. Unfortunately, also the longest brush P(MeOx$_7$-MA)$_{820}$ could not be analyzed by SEC-MALLS due to low amount of polymer product after the work up procedure, which had to be performed to quantitatively remove remaining macromonomer and the catalyst. A typical SEC-MALLS trace along with RI detection of P(MeOx$_7$-MA)$_{52}$ is shown in Figure 4. The determined molar masses are in good agreement with the theoretical values. For most cases, the deviation of the determined number average molar

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masses from the theoretical values is only about 10 % and thus within the experimental error of the method (temperature dependence of d*n*/d*c* and underestimation of species with low scattering intensity). The determined molar masses for the longest molecular brushes ($P(MeOx_7\text{-}MA)_{202}$ and $P(EtOx_{21}\text{-}MA)_{183}$) deviate by about 30 % which cannot be accounted by an uncontrolled ATRP conversion as the dispersity of the products is still very low. One reason could be the strong hygroscopic nature of the used macromonomers which made it difficult to adjust the correct $[M]_0$:$[I]_0$ ratio. However, during attempts to additionally freeze-dry the educts prior use, considerable autopolymerization of the macromonomers were observed which forced us to refrain from additional drying steps. The SEC-MALLS elugram in Figure 4a shows an additional distribution at very high molar masses which is not appearing in the refractive index detection. Similar elugrams with high molar mass distributions but of strongly varying intensities were also observed for other $P(MeOx_7\text{-}MA)_n$ molecular brushes and are attributed to very large metastable brush aggregates. First dynamic light scattering measurements in methanol (data not shown) revealed only one particle distribution assigned to the molecular brushes, underlining the assumption of aggregates only stabilized by hydrophobic interactions in aqueous solution.

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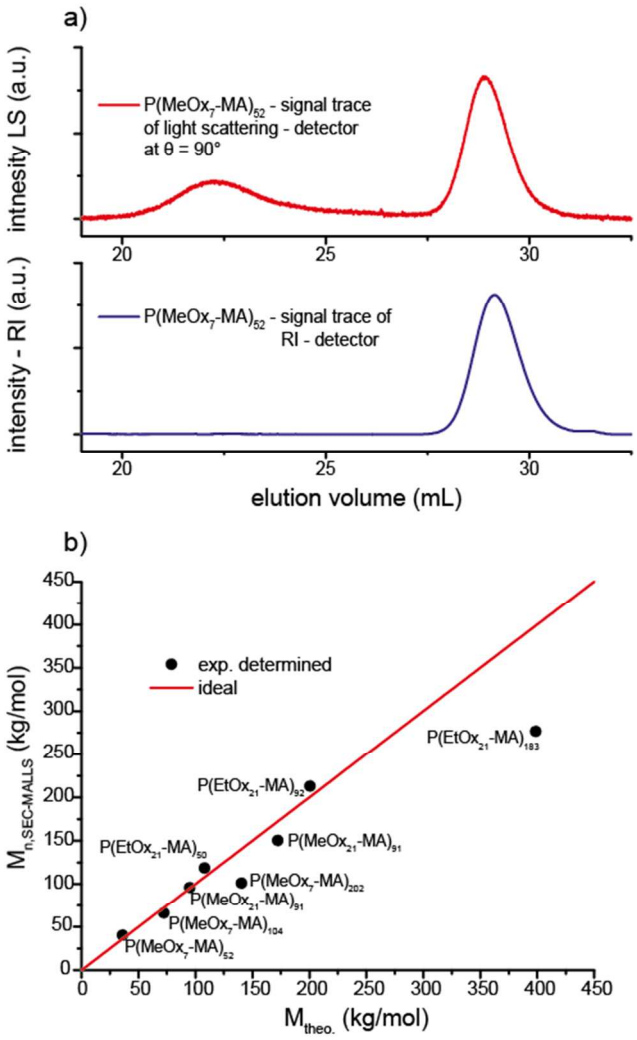

Figure 4. a) SEC-MALLS elugram of P(MeOx$_7$-MA)$_{52}$ (H_2O, 0.72 g/L NaN_3 and 8.5 g/L $NaNO_3$, 35 °C) in red along with SEC-RI detection (blue). The distribution of the molecular brushes is visible at elution volumes between 27.5 to 31 mL with a narrow and symmetrical appearance. The second high molar mass peak is attributed to metastable brush aggregates. b) Comparison of the determined number average molar masses with the theoretical values as calculated from the initial macromonomer/initiator ratio and the conversion. Ideal behavior is indicated by the red line. Only P(MeOx$_7$-MA)$_{202}$ and P(EtOx$_{21}$-MA)$_{183}$ deviate noticeably.

Investigation of the polymerization kinetics of MeOx$_7$-MA as the POx analog to the frequently used OEGMA$_{475}$ revealed a very similar polymerization behavior of both macromonomers (Figure 5) under comparable conditions. For MeOx$_7$-MA as well as for OEGMA$_{475}$ a similar deviation from ideal pseudo-first order kinetics is observable.[58] ATRP of MeOx$_7$-MA resulted in a linear increase of the molar mass as a function of the polymerization time at low dispersities ($Đ \leq 1.18$) even at high conversions.

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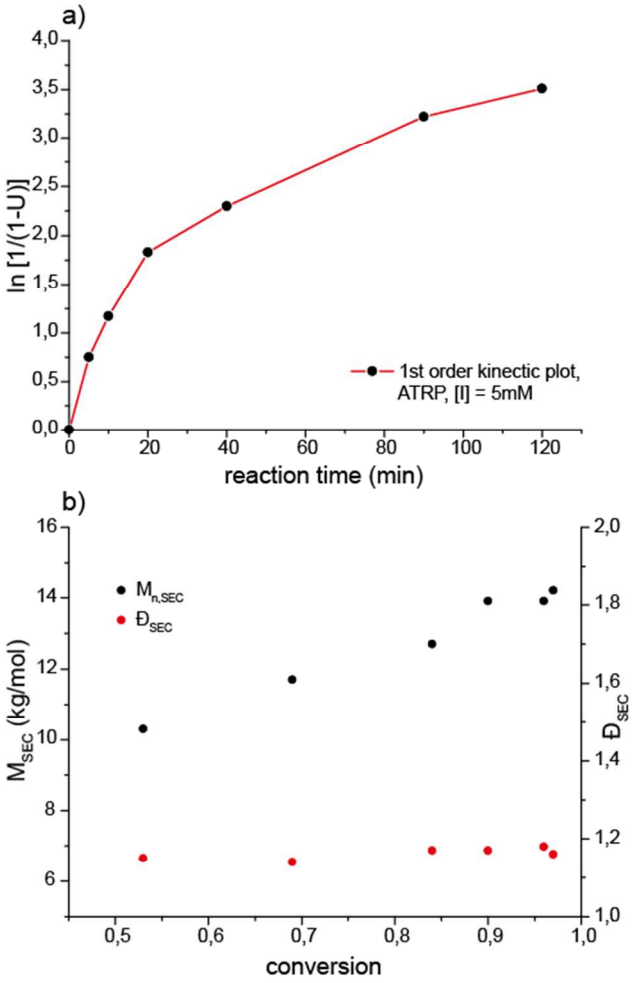

Figure 5. a) First order kinetic plot for the synthesis of molecular brushes based on poly(2-methyl-2-oxazoline) at room temperature in aqueous solution. Initiator concentration [I] = 5 mM; [macromonomer]:[I]:[Cu^{I}]:[Cu^{II}]:[bpy] = 50:1:1:9:22. b) Development of $M_{n,SEC}$ and $Đ_{SEC}$ with the conversion.

Chain extension of a molecular brush was successfully performed and further corroborates the assumption of a controlled polymerization of POx macromonomers without significant loss of chain end functionality. First, $MeOx_{21}$-MA was polymerized to a conversion of > 90 %, an aliquot of the reaction solution was collected and analyzed and the remaining solution further converted *in situ* by addition of $EtOx_{21}$-MA to give a P[($MeOx_{21}$-MA)-($EtOx_{21}$-MA)] block copolymer brush. SEC analysis of the products reveals very low dispersities of both polymers, especially for the block copolymer with a complete shift to a higher molar mass with no indication of residual homopolymer brush (Figure 6). Even though, the remaining $MeOx_{21}$-MA participates during in the second polymerization, this indicates high end group fidelity and

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demonstrates the possibility to prepare block copolymer molecular brushes by the grafting through of POx macromonomers by aqueous ATRP.

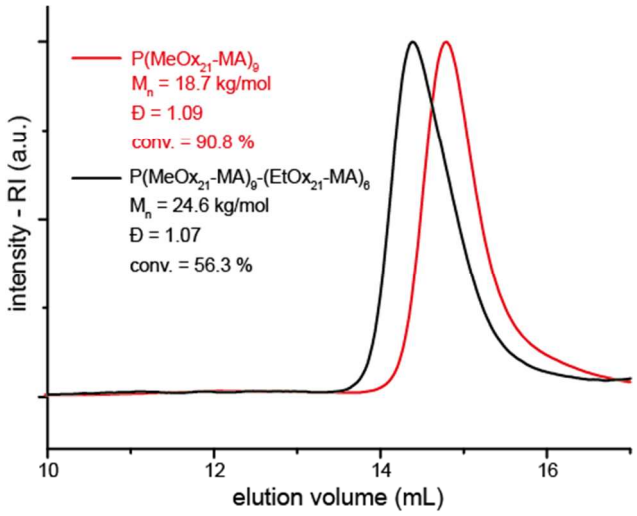

Figure 6. SEC elugrams of the two reaction stages of the chain extension. First, the brush was synthesized by aqueous ATRP of $MeOx_{21}$-MA with initiator concentration [I] = 10 mM; $[MM]/[I]/[Cu^{I}]/[Cu^{II}]/[bpy]$ = 10/1/1/9/22. After 1.5 h a part of the reaction mixture was transferred into a solution of $EtOx_{21}$-MA for in situ chain extension. SEC traces demonstrate a clear shift of the molar mass and the dispersity remains low.

Thermoresponsive behavior

The synthesized molecular brushes based on poly(2-ethyl-2-oxazoline) and poly(2-isopropyl-2-oxazoline) should exhibit a thermoresponsive behavior similar to earlier observations.[48,50,51,53–56] The transmittance of aqueous solutions (1 wt%) of the brushes was observed in dependency of the temperature and the cloud point (T_{CP}) was determined at 10 % decrease in optical transmittance (Figure 7). All four brushes showed very sharp transitions that were in a range of only 1-1.5 K. During several consecutive cooling/heating cycles, the materials showed no deviation of the T_{CP} and only minor heating-cooling hysteresis of approximately 1 K as shown for $P(EtOx_{21}\text{-}MA)_{50}$ and $P(i\text{PrOx}_{23}\text{-}MA)_{37}$ in Figure 7. The determined cloud points appeared in the expected range of T_{CP} = 73°C for $P(EtOx_{21}\text{-}MA)_{50}$ and T_{CP} = 36°C for $P(i\text{PrOx}_{23}\text{-}MA)_{37}$. Increase of the molar mass of $P(EtOx_{21}\text{-}MA)_n$ resulted in the expected slight decrease of the T_{CP}.[48,51,55] The phase transition of $P(i\text{PrOx}_{23}\text{-}MA)_{37}$ is intriguingly close to the human body temperature and falls nicely into the row of previously determined T_{CP}'s of $P(i\text{PrOx}_m)_n$ molecular brushes as a brush made by consecutive living anionic polymerization (backbone) and LCROP

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(grafting from of the side chains) with n = 52, 132, 216 and m = 24 showed a T_{CP} =31, 29.1 and 27°C, respectively.[55]

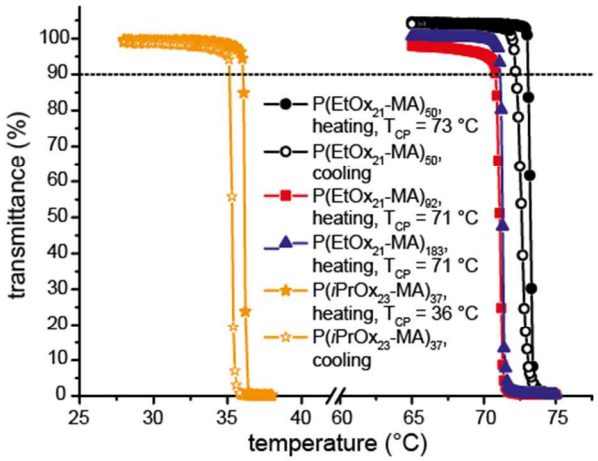

Figure 7. Turbidity measurements for the thermoresponsive brushes in aqueous solution (1 wt%). Heating/cooling rate was 1 K/min. For optical clarity, cooling traces are only shown for P(EtOx$_{21}$-MA)$_{50}$ and P(*i*PrOx$_{23}$-MA)$_{37}$, but heating-cooling hysteresis was small (~ 1 K) for all brushes. T_{CP} was determined at 10 % decrease in transmittance on heating.

Conclusion

A series of molecular brushes of poly(2-oxazoline)s was synthesized by grafting through polymerization of oligo- and poly(2-oxazoline) macromonomers by aqueous ATRP at room temperature. These molecular brushes feature high grafting densities, narrow molar mass distributions and adjustable molar masses. It was found that surprisingly long and high molar mass molecular brushes are accessible by the grafting through approach, however, the method is limited by the initial viscosity of the reaction solution. Oligo(2-methyl-2-oxazoline)methacrylate showed a very similar polymerization behavior as the commercially available OEGMA$_{475}$ including the deviation from first order kinetics as well as low dispersities and almost linear increase of the molar mass with the ATRP reaction time. Thus, a versatile POx-based macromonomer to OEGMA$_{475}$ is now available for the synthesis of structurally complex biomaterials. Chain extension and formation of block copolymer molecular brushes were also successful. Aqueous solutions of POx molecular brushes with poly(2-ethyl- and 2-isopropyl-2-oxazoline) side chains exhibit the typically defined thermoresponsive behavior with a tunable, very narrow and reversible phase transition. The T_{CP} of P(*i*PrOx$_{23}$-MA)$_{37}$ lays with 36°C close to human body temperature and falls nicely into the row of previously reported P(*i*PrOx$_m$)$_n$ molecular brushes synthesized by grafting from and living ionic polymerization. In conclusion,

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aqueous ATRP of oligo- and poly(2-oxazoline)methacrylates was found to be a versatile synthetic route for the preparation of defined POx molecular brushes.

Acknowledgements

Financial support by the "Excellence Initiative by the German Federal and State Governments" (Institutional Strategy, measure "support the best") is gratefully acknowledged.

Experimental

Materials and Methods

All chemicals were purchased from Sigma-Aldrich (Steinheim, Germany) or Acros Organics (Geel, Belgium) and used as received if not stated otherwise. 2-Methyl-, 2-ethyl-, 2-isopropyl-2-oxazoline, methyltriflate and triethylamine were dried by refluxing over calcium hydride and distillation under a dry nitrogen atmosphere. 2-Isopropyl-2-oxazoline was synthesized as described elsewhere.[64] Methacrylic acid was distilled *in vacuo* to remove the inhibitor and stored under dry nitrogen atmosphere at 7 °C. Aqueous solutions were prepared with deionized water (Millipore, 18.2 MΩcm) if not stated otherwise.

^{1}H-NMR-spectroscopy was performed on a DRX 500 P or a AC 300 P (Bruker, Germany) and spectra were calibrated to the solvent residual signal. MALDI-ToF-MS was performed on a Bruker Biflex (Bruker Daltonics, Bremen, Germany) with a N_2-Laser ($\lambda = 337$ nm) in positive reflector mode. Spectra were recorded in a range from 40 to 4400 Da and the matrix was suppressed up to 330 Da ($MeOx_7$-MA), 450 Da ($EtOx_{21}$-MA, $IPOx_{23}$-MA) or 500 Da ($MeOx_{21}$-MA), respectively. The device was calibrated from 750 to 3150 Da with Peptide Calibration Standard II (Bruker) prior to every measurement. Samples were prepared by the dried droplet method. Macromonomers were dissolved at 1 g/L in methanol with 1 % trifluoroacetic acid and subsequently mixed in a ratio of 5:1 (v/v) with the matrix (20 g/L sinapinic acid in methanol with 1 % trifluoroacetic acid). Turbidity measurements were performed on a Lambda 35 UV/VIS spectrometer equipped with a PTP-1 Peltier System (all Perkin Elmer, Germany) and controlled using Templab software provided by the instrument supplier. The T_{CP} of the molecular brushes were determined by spectrophotometric detection of the changes in transmittance at $\lambda = 500$ nm of 1.0 wt% aqueous solutions. The heating/cooling rate was 1.0 K/min. Given values for the T_{CP} were determined as the temperature corresponding to a 10% decrease in optical transmittance.

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Size exclusion chromatography (SEC) was done on a PL-GPC 120, equipped with two Gram 100 10 µL 8x300 mm columns and dimethylacetamide (DMAc) with 5 g/L LiBr and 1 vol% H_2O as the mobile phase at 70 °C. The system was calibrated with PMMA standards (PSS, Mainz, Germany) and RI detection. Samples were dissolved in the mobile phase and filtered through 0.2 µm PTFE syringe filters prior to the measurement. SEC-MALLS was performed on a system from Jasco (Gross-Umstadt, Germany) with a PU 2080 HPLC-pump, a Jet-Stream II Plus column oven equipped with one SUPREMA 10 µm 100 Å 8x300 mm column and two SUPREMA 10 µm 3000 Å 8x300 mm columns (all from PSS). As the mobile phase, double distilled water with 0.72 g/L NaN_3 and 8.5 g/L $NaNO_3$ was used at a temperature of 35 °C. A Dawn DSP laser photometer at λ = 632.8 nm (Wyatt Technology, Dernbach, Germany) and a RI-930 RI detector (Jasco) was used for detection. Samples were dissolved in the mobile phase and filtered through 0.22 µm CME-filters prior to the measurement. Refractive index increments d*n*/d*c* were determined on a differential refractometer DR1/b from SLS Systemtechnik (Denzlingen, Germany) in a concentration range of 1 to 5 g/L at a temperature of 25 °C. Polymers were weighed immediately after lyophilization and dissolved in double distilled water containing 0.72 g/L NaN_3 and 8.5 g/L $NaNO_3$. Results of the d*n*/d*c* measurement can be found in Table 4.

Tab 4. Results of the d*n*/d*c* measurements with the molecular brushes.

Name	d*n*/d*c* (mL/g)
$P(MeOx_7\text{-}MA)_{52}$	0.118
$P(MeOx_7\text{-}MA)_{104}$	0.116
$P(MeOx_7\text{-}MA)_{202}$	0.114
$P(MeOx_{21}\text{-}MA)_{50}$	0.146
$P(MeOx_{21}\text{-}MA)_{91}$	0.153
$P(EtOx_{21}\text{-}MA)_{50}$	0.149
$P(EtOx_{21}\text{-}MA)_{92}$	0.156
$P(EtOx_{21}\text{-}MA)_{183}$	0.153

Macromonomer synthesis

Macromonomer $MeOx_7$-MA: In a glove box, 5.7774 g (35.2 mmol, 1 eq) methyltriflate was dissolved in 45 mL acetonitrile and 14.9237 g (175.4 mmol, 5.0 eq) 2-methyl-2-oxazoline were added slowly at r.t.. The solution was stirred at 90 °C for 20 min. Afterwards the solution was

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cooled to r.t. and 7.6507 g (88.9 mmol, 2.5 eq) methacrylic acid and 8.9936 g (88.9 mmol, 2.5 eq) triethylamine were added. The solution was stirred at 70 °C over night. After cooling to r.t. approx. 3 g K_2CO_3 were added and the mixture was stirred for 24 h. The resulting suspension was centrifuged, the solution decanted and filtered through a 20 µm PTFE syringe filter. The solvent was removed *in vacuo* and the residual solid was cleaned by three-fold precipitation from methanol in a 10-fold excess of ice-cold dry diethyl ether. After several hours of drying under reduced pressure the macromonomer was obtained as a slightly yellow powder (11.7125 g, 63 %). ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.92 (s, 3 H, C*H*$_3$-C(=CH$_2$)-), 2.10 (br, 21 H, C*H*$_3$-CO-), 2.95 and 3.05 (m, 3 H, C*H*$_3$-N-), 3.54 (br, 26 H, -N-C*H*$_2$-C*H*$_2$-), 4.27 (br, 2 H, -CH$_2$-C*H*$_2$-O-), 5.60 (s, 1 H, -C=C*H*$_2$), 6.08 (s, 1 H, -C=C*H*$_2$); $M_{n,NMR}$ = 695 g/mol, f_{NMR} = 1. SEC: $Đ_{SEC}$ = 1.31, $M_{n,SEC}$ = 424 g/mol. MALDI-ToF-MS: $Đ_{MALDI}$ = 1.04, $M_{n,MALDI}$ = 524 g/mol.

Macromonomer MeOx$_{21}$-MA: MeOx$_{21}$-MA was obtained as described above using 0.9668 g (5.9 mmol, 1 eq) methyltriflate in 30 mL acetonitrile, 10.0592 g (118.2 mmol, 20.0 eq) 2-methyl-2-oxazoline, 1.3253 g (15.4 mmol, 2.6 eq) methacrylic acid and 1.5622 g (15.4 mmol, 2.6 eq) triethylamine. Polymerization time was 30 min, termination occurred over night. The macromonomer was received as a slightly yellow powder (8.9415 g, 84 %). ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.92 (s, 3 H, C*H*$_3$-C(=CH$_2$)-), 2.09 (br, 63 H, C*H*$_3$-CO-), 2.94 and 3.04 (m, 3 H, C*H*$_3$-N-), 3.51 (br, 82 H, -N-C*H*$_2$-C*H*$_2$-), 4.27 (br, 2 H, -CH$_2$-C*H*$_2$-O-), 5.60 (s, 1 H, -C=C*H*$_2$), 6.08 (s, 1 H, -C=C*H*$_2$); $M_{n,NMR}$ = 1887 g/mol, f_{NMR} = 1. SEC: $Đ_{SEC}$ = 1.09, $M_{n,SEC}$ = 2010 g/mol. MALDI-ToF-MS: $Đ_{MALDI}$ = 1.02, $M_{n,MALDI}$ = 1790 g/mol.

Macromonomer EtOx$_{21}$-MA: As described above, EtOx$_{21}$-MA was obtained using 0.7705 g (4.7 mmol, 1 eq) methyltriflate in 30 mL acetonitrile, 9.3522 g (94.3 mmol, 20.1 eq) 2-ethyl-2-oxazoline, 1.0241 g (11.9 mmol, 2.5 eq) methacrylic acid and 1.1956 g (11.8 mmol, 2.5 eq) triethylamine. Polymerization time was 90 min, termination was performed over night. The macromonomer was received as a white powder (7.9394 g, 81 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.12 (br, 63 H, C*H*$_3$-CH$_2$-CO-), 1.91 (s, 3 H, C*H*$_3$-C(=CH$_2$)-), 2.36 (br, 42 H, CH$_3$-C*H*$_2$-CO-), 2.95 and 3.02 (m, 3 H, C*H*$_3$-N-), 3.50 (br, 82 H, -N-C*H*$_2$-C*H*$_2$-), 4.26 (br, 2 H, -CH$_2$-C*H*$_2$-O-), 5.58 (s, 1 H, -C=C*H*$_2$), 6.06 (s, 1 H, -C=C*H*$_2$); $M_{n,NMR}$ = 2182 g/mol, f_{NMR} = 1. SEC: $Đ_{SEC}$ = 1.09, $M_{n,SEC}$ = 2484 g/mol. MALDI-ToF-MS: $Đ_{MALDI}$ = 1.02, $M_{n,MALDI}$ = 2050 g/mol.

Macromonomer iPrOx$_{23}$-MA: As described above *i*PrOx$_{23}$-MA was obtained using 0.7238 g (4.5 mmol, 1 eq) methyltriflate in 30 mL acetonitrile, 10.2399g (90.5 mmol, 20.1 eq) 2-isopropyl-2-oxazoline, 0.9690 g (11.3 mmol, 2.5 eq) methacrylic acid and 1.1388 g (11.3 mmol, 2.5 eq)

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triethylamine. Polymerization time was 120 min, termination occurred over night. The macromonomer was received as a white powder (5.1543 g, 55 %). ^{1}H-NMR (300 MHz, MeOD): δ (ppm) = 1.20 (br, 138 H, (C*H*$_3$)$_2$-CH-), 2.00 (s, 3 H, C*H*$_3$-C(=CH$_2$)-), 2.84 and 3.08 (br, 26 H, C*H*$_3$-N- and (CH$_3$)$_2$-C*H*-), 3.70 (br, 90 H, -N-C*H*$_2$-C*H*$_2$-), 4.42 (br, 2 H, -CH$_2$-C*H*$_2$-O-), 5.74 (s, 1 H, -C=C*H*$_2$), 6.16 (s, 1 H, -C=C*H*$_2$); $M_{n,NMR}$ = 2702 g/mol, f_{NMR} = 1. SEC: $Đ_{SEC}$ = 1.08, $M_{n,SEC}$ = 3063 g/mol. MALDI-ToF-MS: $Đ_{MALDI}$ = 1.02, $M_{n,MALDI}$ = 2450 g/mol.

ATRP of MeOx$_7$-MA with different ligands and Cu(II) ratio

In general MeOx$_7$-MA, 2-hydroxyethyl-2-bromoisobutyrat, and the ligand were dissolved in 2 mL of water in a small tube in the desired ratio. The tube was sealed with a rubber septum and the solution was degassed using a nitrogen flow for 30 min. CuBr and CuBr$_2$ were filled into another tube, sealed and evacuated/purged with dry nitrogen (3 times). Subsequent the macromonomer, initiator, ligand solution was transferred to the copper salts using a gastight syringe. The reaction mixture was stirred for 2 h at r.t. and after that diluted with water and lyophilized. The resulting colored powders were analyzed by SEC. For further details refer to Table 2.

Synthesis of molecular brushes (P(Ox$_m$-MA)$_n$)

Molecular brushes of MeOx$_7$-MA (P(MeOx$_7$-MA)$_n$): In a typical run for the synthesis of P(MeOx$_7$-MA)$_{10}$ 72.6 mg (0.1045 mmol, 10.4 eq) MeOx$_7$-MA were dissolved in 2 mL of a 5 mM aqueous solution of the initiator 2-hydroxyethyl-2-bromoisobutyrat (2.1105 mg, 0.01 mmol, 1 eq) in a small tube and sealed with a rubber septum. The solution was degassed using a nitrogen flow for 30 min. Meanwhile 1.5 mg (0.0105 mmol, 1.1 eq) CuBr, 20.5 mg (0.0918 mmol, 9.2 eq) CuBr$_2$ and 34.6 mg (0.2215 mmol, 22.2 eq) bpy were weighed in another tube and sealed with a rubber septum. Subsequent the tube was carefully evacuated and backfilled with nitrogen three times. Afterwards the macromonomer-initiator solution was transferred to the solid components using a gas-tight syringe. The resulting mixture was stirred at r.t. After 2 h the reaction was stopped by dilution with oxygen containing *Millipore* water and the solution was lyophilized. A small sample was taken from the obtained blue powder to determine the conversion by SEC. The residual solid was dissolved in *Millipore* water again and passed over a short silica column to remove the catalyst and residual macromonomer. The clear, colorless solution was lyophilized once more and the molecular brush was obtained as a slight yellowish powder (41.9 mg, 56 %).

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Conversion was determined from the ratio of the macromonomer- to molecular brush- band-area in the SEC-traces. conv. >99 %. ^{1}H-NMR (500 MHz, DMSO-d_6): δ (ppm) = 1.04 (br, 4 H, (CH_3)$_2$C- and -CH$_2$-C(CH_3)-CH$_2$-), 1.90 (br, 23 H, -CH_2-C(CH$_3$)- and CH_3-CO-), 2.80 (d) and 2.94 (dd) (3 H, CH_3-N-), 3.36 (br, 60 H, -N-CH_2-CH_2- and water). SEC: $Đ_{SEC}$ = 1.16, $M_{n,SEC}$ = 6.9 kg/mol.

Molecular brushes of MeOx$_{21}$-MA (P(MeOx$_{21}$)$_m$): Molecular brushes of MeOx$_{21}$-MA were synthesized according to the procedure described above using MeOx$_{21}$-MA as macromonomer. ^{1}H-NMR (500 MHz, DMSO-d_6) exemplary for P(MeOx$_{21}$-MA)$_{50}$: δ (ppm) = 1.04 (br, 3 H, (CH_3)$_2$C- and -CH$_2$-C(CH_3)-CH$_2$-), 1.92 (br, 60 H, -CH_2-C(CH$_3$)- and CH_3-CO-), 2.80 (d) and 2.94 (dd) (3 H, CH_3-N-), 3.35 (br, 84 H, -N-CH_2-CH_2- and water).

Molecular brushes of EtOx$_{21}$-MA: Molecular brushes of EtOx$_{21}$-MA were synthesized according to the procedure described above using EtOx$_{21}$-MA as macromonomer. ^{1}H-NMR (500 MHz, DMSO-d_6) exemplary for P(EtOx$_{21}$-MA)$_{50}$: δ (ppm) = 1.12 (br, 63 H, CH_3-CH$_2$-CO-), 2.19 (br, 40 H, CH$_3$-CH_2-CO-), 2.81 (s) and 2.94 (d) (3 H, CH_3-N-), 3.35 (br, 91 H, -N-CH_2-CH_2- and water).

*Molecular brush of *i*PrOx$_{23}$-MA (P(*i*PrOx$_{23}$-MA)$_n$)*: The molecular brush of *i*PrOx$_{23}$-MA was synthesized according to the procedure described above using *i*PrOx$_{23}$-MA as macromonomer. ^{1}H-NMR (500 MHz, DMSO-d_6) P(*i*PrOx$_{23}$-MA)$_{37}$: δ (ppm) = 0.96 (br, 138 H, (CH_3)$_2$-CH-), 2.12 (br, 2 H, -CH_2-C(CH$_3$)-), 2.78 (br, 27 H, (CH$_3$)$_2$-C*H*-), 3.00 (d, 3 H, CH_3-N-), 3.35 (br, 102 H, -N-CH_2-CH_2- and water).

Interestingly, the work-up procedure for all molecular brushes was facile as the residual macromonomers as well as the remaining catalyst could be easily and quantitatively removed by column chromatography.

Characterization of molecular brushes was performed by SEC, SEC-MALLS and ^{1}H-NMR spectroscopy. For a summary of the experimental conditions and analytical data please refer to Table 3.

Kinetic investigations

The sample for the kinetic investigation was prepared as described above for the synthesis of the molecular brushes. Initiator concentration was 5 mmol/L, MeOx$_7$-MA was used as macromonomer and the reaction was conducted at r.t.. Samples of 0.05 mL were taken periodically under inert atmosphere at t = 0, 5, 10, 20, 40, 60, 90 and 120 min and immediately freeze dried. Conversion, $M_{n,SEC}$ and $Đ_{SEC}$ were determined for every sample using SEC.

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Chain extension experiments

In a glass tube, 198.7 mg (0.1053, 10.5 eq) $MeOx_{21}$-MA was dissolved in 1 mL of a 10 mM aqueous solution of 2-hydroxyethyl-2-bromoisobutyrat (2.1105 mg, 0.010 mmol, 1 eq). The tube was sealed with a rubber septum and the solution was purged with nitrogen for 30 min. Meanwhile, 1.4 mg (0.0098 mmol, 1 eq) CuBr, 20.1 mg (0.0899 mmol, 9.0 eq) $CuBr_2$ and 34.7 mg (0.2222 mmol, 22.2 eq) bpy were filled into a tube, sealed and evacuated/purged with dry nitrogen (3 times). Subsequently, the macromonomer-initiator solution was added to the solid components with a gas tight syringe under Schlenk conditions. The reaction mixture was stirred for 1.5 h at r.t.. An aliquot of 0.1 mL was taken to determine conversion, molar mass and dispersity of the first reaction step (conv. = 90.8 %, $M_{n,SEC}$ = 18.7 kg/mol, $Đ_{SEC}$ = 1.09). From the remaining solution, 0.8 mL were transferred to a degassed solution of 199.1 mg (0.0912 mmol, 11.4 eq) $EtOx_{21}$-MA in 0.9 mL water. Immediately afterwards a sample of 0.1 mL was taken. The remaining mixture was stirred for another 3 h. The work-up procedure was as described above. The copolymer brush was obtained as a white powder (183.5 mg, 51 %). conv. = 56.3 %. ^{1}H-NMR (500 MHz, DMSO-d_6): δ (ppm) = 0.95 (br, 19 H, CH_3-CH_2-CO-), 1.91 (30 H, -CH_2-C(CH_3)- and CH_3-CO-), 2.29 (br, 12 H, CH_3-CH_2-CO-), 2.81 (m) and 2.94 (d) (3 H, CH_3-N-), 3.34 (br, 73 H, -N-CH_2-CH_2- and water). SEC: $Đ_{SEC}$ = 1.07, $M_{n,SEC}$ = 24.6 kg/mol.

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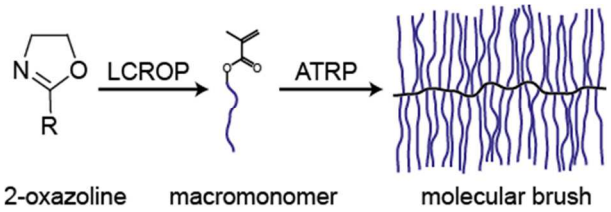

Well defined molecular brushes of poly(2-oxazoline)s were synthesized by ATRP of oligo- and poly(2-methyl-, 2-ethyl- and 2-isopropyl-2-oxazoline) macromonomers in aqueous solution.

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